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IL-6, IL-8 and IL-10 Levels in Healthy Weight and Overweight Children

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Key Words
Cytokine · Inflammation · Obesity · Children · Interleukin-8

Abstract
Background/Aim: In adults, studies have shown that obesity is a chronic low-grade inflammatory state characterized by altered levels of cytokines. Studies in children have mainly focused on C-reactive protein and adiponectin, and there is limited data for other inflammatory markers in healthy weight and overweight children. The aim of this study was to measure IL-6, IL-8 and IL-10 levels in healthy normal weight and overweight children at 8 and 15 years. ***Methods:*** 118 normal weight and overweight children (59 boys) from the Nepean longitudinal study were recruited at age 8 years and followed up at 15 years. Serum IL-6, IL-8 and IL-10 levels were measured at both time-points. ***Results:*** At 8 years, we found no significant differences in cytokine levels between normal weight and overweight (owt)/obese (ob) groups. However, at 15 years, owt/ob girls (n = 23) had higher levels of IL-6 (p = 0.04), IL-8 (p = 0.04) and IL-10 (p = 0.03) compared to normal weight girls (n = 36), even after adjustment for puberty; no differences were seen in boys. ***Conclusion:*** The effects of obesity on IL-6, IL-8 and IL-10 levels vary with age and sex, with owt/ob girls at 15 years showing raised IL-6, IL-8 and IL-10 levels compared to healthy weight girls.

Introduction

In adults, it is well established that obesity is a state of chronic low-grade inflammation. This is evidenced by the moderate increases in circulating levels of proinflammatory cytokines (or decreasing levels in the case of adiponectin) in obese adults and histopathological studies showing macrophage infiltration in adipose tissue from obese adults [1]. Dysregulation of cytokine levels may contribute to the pathogenesis of insulin resistance and diabetes, and subclinical inflammation is a key element in the development of atherosclerosis [2, 3].

While the relation between obesity and both raised C-reactive protein (CRP) [4, 5] and decreased adiponectin (including high-molecular-weight adiponectin) levels [6–10] in children is well documented, studies examining IL-6 levels in overweight children show conflicting findings [8, 11–13]. Obese prepubertal Turkish children have significantly elevated IL-6 levels compared to lean controls [11], and Herder et al. [12] showed a significant association between IL-6 levels and BMI (adjusted for age, sex and lipids) in a large cohort of German adolescents. In contrast, two other studies have shown no differences in IL-6 levels between lean and obese groups in Spanish children aged 6–9 years [13] and Hispanic children aged 10–18 years [8]. IL-8, a proinflammatory cytokine, is elevated in obese adults [14, 15] and has been implicated in

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the pathogenesis of atherosclerosis, with elevated IL-8 levels in healthy adults with an increased risk of cardiovascular disease [16]. To date, there has only been one study measuring IL-8 in healthy weight and overweight adolescents which found no association between IL-8 and BMI [12]. To our knowledge, there have been no studies measuring IL-10, an anti-inflammatory cytokine, in healthy weight and overweight children and no studies examining longitudinal changes in IL-6, IL-8 and IL-10 levels in the pediatric population.

The aim of this study was to examine associations between IL-6, IL-8 and IL-10 levels and weight status in a longitudinal cohort of healthy children at age 8 and 15 years.

Methods

Subjects

In 1996/1997, 436 healthy 8-year-old children (215 girls) were recruited for the longitudinal Nepean study which was designed to investigate the effect of birth size, body size and genes on blood pressure and bone mass. All were born at term at the Nepean Hospital, Penrith, in western Sydney between August 1989 and April 1990, and were part of a birth cohort whose details and selection criteria have been previously published [17, 18]. The children were predominantly (>96%) of European descent, and 260 children (out of a total of 436) agreed to have a blood sample taken.

Between July 2004 and March 2005, the children were recontacted, and 290 (67.2%) agreed to participate in the follow-up and 172 (39% of the original cohort) agreed to have a blood sample taken. 118 participants (59 boys) with paired blood samples collected at age 8 and 15 years were included in this study. At 8 and 15 years, there were no significant differences in weight, BMI and waist circumference (both raw data and z-scores), height z-score and the number of children classified as overweight/obese, between children that were followed up (n = 118) and those that were not (n = 318). Written consent was obtained from their parents and the Ethics Committees of The Children's Hospital at Westmead and Western Sydney Area Health Service approved the study.

Anthropometry and Pubertal Assessment

Anthropometry was measured at age 8 and 15 years. Height was measured to the nearest 0.1 cm and weight was measured in minimal clothing to the nearest 0.1 kg with electronic scales. Waist circumference was measured to the nearest 0.1 cm with a flexible steel tape at the level of the narrowest point between the lower costal border and the iliac crest. z-Scores from age- and sex-specific reference values were calculated for BMI [19] and waist circumference [20]. The International Obesity Taskforce (IOTF) BMI cut-points were used to define overweight (owt) and obesity (ob) [21].

At 8 years, puberty was not formally assessed, but the participants were viewed in undergarments during anthropometric assessment and none was overtly pubertal. At 15 years, pubertal status was determined using self-assessed Tanner staging of breast development (girls) and genitalia (boys) [22]. Girls also reported if and when they commenced menses. For analysis, young people were categorized as prepuberty (Tanner stage 1), early puberty (Tanner stages 2 and 3) and late puberty (Tanner stages 4 and 5).

Biochemistry

Fasting blood samples were collected by venipuncture and glucose levels were measured using the glucose oxidase method. Serum samples were frozen at –80°C until analyzed for insulin, IL-6, IL-8 and IL-10. Insulin was measured using a commercial kit (Linco Research, Inc., St. Charles, Mo., USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by dividing the product of insulin (mU/ml) and glucose (mmol/l) by 22.5 [23]. HOMA-IR method is a validated marker of insulin resistance in children [24, 25].

IL-6, IL-8 and IL-10 levels were determined using a multiplex, bead-based cytokine detection method (BioSource International, Camarillo, Calif., USA) with detection limits of 0.24 pg/ml for IL-6, 0.37 pg/ml for IL-8 and 0.12 pg/ml for IL-10. Intraassay coefficients for IL-6, IL-8 and IL-10 were 6.6, 7.2 and 9.4%, and interassay coefficients were 7, 9.8 and 9.8%, respectively. TNF-α levels were also measured, but levels were undetectable due to technical difficulties. Cytokine measures were obtained on the Biorobot 3000 instrument integrated with the Liquichip Workstation (Qiagen, Valencia, Calif., USA). All samples were assayed at the end of data collection, and assay protocol followed the kits' instructions.

Statistical Analysis

All analyses were conducted using the SPSS statistical package (SPSS for Windows, Version XV, Chicago, Ill., USA), and p values reported are two-tailed with statistical significance set at <0.05. A χ^2 test (using continuity correction) was used to analyze group sex differences, independent sample t tests used to compare normally distributed data (height, weight, BMI, BMI z-score, waist circumference and waist circumference z-score) and Mann-Whitney U test for not normally distributed data (IL-6, IL-8, IL-10 and HOMA-IR). Cytokine values at 15 years were $\log^{10}$ transformed and analysis of covariance used to examine differences between normal weight and owt/ob groups with pubertal status as a covariate.

To examine associations between BMI changes from 8 to 15 years and cytokine levels at 15 years, participants were grouped using IOTF cut-points: (1) acceptable BMI at baseline and follow-up, (2) BMI gainers – acceptable BMI at baseline, but owt/ob at follow-up, (3) BMI losers – owt/ob at baseline, but acceptable BMI at follow-up, and (4) owt/ob at both time-points. Differences in continuous variables between BMI groups were compared using the Kruskal-Wallis test. For post-hoc analysis, the Mann-Whitney U test was used to compare differences between BMI groups. To examine associations between cytokine levels and cardiovascular risk clustering at 15 years, children were grouped as having presence/absence of cardiovascular risk factors, as previously described [26]. Cardiovascular risk clustering was described as three or more of the following: fasting glucose ≥6.1 mmol/l; triglycerides ≥80th centile of the study population; systolic blood pressure ≥90th centile for age, sex and height centile [27], and overweight or obese.

Table 1. Participant characteristics at 8 and 15 years of age

	8 years			15 years		
	normal weight	overweight/obese	p value	normal weight	overweight/obese	p value
Participants, n	90 (50 boys)	28 (9 boys)	0.051[a]	75 (39 boys)	43 (20 boys)	0.699[a]
Age, years	7.9 ± 0.6	7.7 ± 0.8	0.015[b]	14.9 ± 0.2	14.9 ± 0.3	0.756[b]
Height, cm	128.4 ± 6.7	128.0 ± 6.7	0.716[b]	167.3 ± 8.8	162.2 ± 8.0	0.486[b]
Weight, kg	26.2 ± 3.7	37.7 ± 7.5	<0.001[b]	55.9 ± 7.3	83.1 ± 10.5	<0.001[b]
BMI	15.8 ± 1.2	22.8 ± 2.0	<0.001[b]	20.0 ± 0.2	31.5 ± 1.9	<0.001[b]
BMI z-score	–0.06 ± 0.75	2.14 ± 0.18	<0.001[b]	–0.03 ± 0.68	2.00 ± 0.20	<0.001[b]
Waist circumference, cm	55.5 ± 3.9	63.2 ± 4.9	<0.001[b]	69.5 ± 4.8	83.9 ± 7.4	<0.001[b]
Waist z-score	–0.30 ± 0.77	1.16 ± 0.64	<0.001[b]	–0.09 ± 0.70	1.60 ± 0.61	<0.001[b]
HOMA-IR	0.8 (0.6–1.2)	1.4 (1.0–1.9)	<0.001[c]	3.0 (2.4–3.8)	3.9 (3.0–4.8)	0.001[c]
IL-6, pg/ml	1.3 (0–2.5)	1.4 (0.2–3.6)	0.313[c]	7.9 (0–9.2)	8.5 (7.1–10.4)	0.037[c]
IL-8, pg/ml	11.6 (5.8–19.5)	10.3 (2.8–19.0)	0.518[c]	18.9 (11.6–27.5)	23.2 (15.0–30.0)	0.011[c]
IL-10, pg/ml	0.6 (0.2–0.9)	0.6 (0.03–1.1)	0.939[c]	7.7 (0.9–7.9)	7.9 (7.3–8.1)	0.023[c]

Data are presented as mean ± SD or median (interquartile range). [a] χ^2 test. [b] Independent samples t test. [c] Mann-Whitney U test.

Results

Participant Characteristics

118 children (59 males) participated in this study at age 8 and 15 years. Participant characteristics and inflammatory markers are shown in table 1. At 8 years, owt/ob children were slightly younger than normal weight children ($p = 0.02$), had higher waist circumferences ($p < 0.001$) and had significantly higher HOMA-IR ($p < 0.001$). 22% (n = 28) of children were classified as owt/ob (32% of girls and 15% of boys). This increased to 37% (n = 44) at 15 years (39% of girls and 34% of boys). At 15 years, 97% (57 of 59) girls had attained menarche and 63% (37/59) of girls were in late puberty (Tanner stage 4 and 5 for breast development); 58% of boys were in late puberty (Tanner stage 4 and 5 for genitalia). For analysis of cytokine levels, the percentages of children with detectable levels for IL-6, IL-8 and IL-10 were 81, 94 and 94%, respectively.

Cytokine Levels

At 8 years, there were no significant differences in IL-6, IL-8 and IL-10 levels between normal weight and owt/ob groups. At 15 years, owt/ob children (n = 43, 20 boys) had higher waist circumferences ($p < 0.001$), HOMA-IR ($p = 0.001$) and levels of IL-6 ($p = 0.04$), IL-8 ($p = 0.01$) and IL-10 ($p = 0.02$) compared to normal weight children. When boys and girls were analyzed separately, we found raised levels of IL-6 [median (interquartile range) = 9.1 (7.9–11.0) pg/ml; $p = 0.04$], IL-8 [26.6 (20.7–31.7) pg/ml; $p = 0.04$] and IL-10 [7.9 (7.5–8.1) pg/ml; $p = 0.03$] in owt/ob girls (n = 23), compared to normal weight girls at age 15 years [IL-6 = 8.5 (0.1–9.6) pg/ml; IL-8 = 8.5 (0.1–9.6) pg/ml (fig. 1a); IL-10 = 7.8 (1.1–7.9) pg/ml]; these differences persisted after adjustment for pubertal status. No significant differences were seen in boys (before and after adjustment for pubertal status). There were no significant correlations between cytokine levels and both BMI and waist circumference (both raw data and z-scores) at either age.

BMI Change Status and Cytokine Levels at 15 Years

Children were categorized into groups based on their BMI at 8 and 15 years. 68 children (38 boys) had an acceptable BMI at 8 and 15 years, 22 children (12 boys) were defined as BMI gainers (acceptable BMI at 8 and owt/ob at 15 years). 7 children (1 boy) were BMI losers (overweight BMI at 8 years and acceptable BMI at 15 years). 21 children (8 boys) were overweight at both time-points (table 2). There were no significant differences between the BMI groups for IL-6 and IL-8; however, with post-hoc testing, we found raised IL-6 ($p = 0.02$), IL-8 ($p = 0.02$) (fig. 1b) and IL-10 ($p = 0.04$) levels in the BMI gainer group compared to the acceptable BMI group. IL-10 levels in the BMI gainer group were significantly higher than the BMI loser group (median = 7.9 vs. 7.5 pg/ml, $p = 0.02$). We did not perform an adjustment for multiple comparisons in these analyses. However, there was no change in the results when LSD/Bonferroni adjustment was performed.

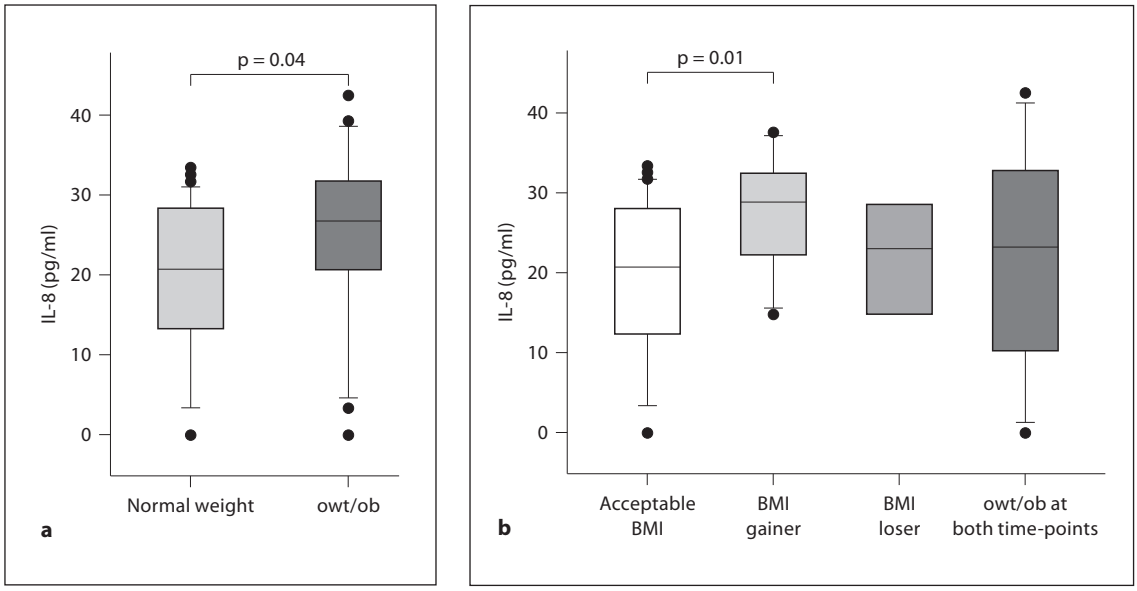

Fig. 1. Serum IL-8 levels in (**a**) normal weight and overweight/obese girls at 15 years, and (**b**) BMI status groups at 15 years (determined using IOTF cut-points [21]). Girls were grouped into (1) acceptable BMI at baseline and follow-up, (2) BMI gainers – acceptable BMI z-score at baseline, but overweight/obese at follow-up, (3) BMI losers – overweight/obese at baseline, but acceptable BMI at follow-up, and (4) overweight/obese at both time-points.

When boys and girls were analyzed separately, we found raised IL-8 and IL-10 levels in girls that gained BMI compared to the girls in the acceptable BMI group (median IL-8 = 9.5 vs. 8.5 pg/ml, p = 0.01; median IL-10 = 8.0 vs. 7.8 pg/ml, p = 0.04).

There were no differences in cytokine levels at 8 and 15 years between children with and without cardiovascular risk clustering at 15 years (data not shown).

Discussion

In this longitudinal study, owt/ob girls at 15 years had higher levels of circulating IL-6, IL-8 and IL-10 compared to normal weight girls, which remained significant after adjustment for pubertal status; no differences were seen in boys. Girls that were normal weight at 8 years and owt/ob at 15 years had raised levels of IL-8 and IL-10 at 15 years compared to girls that were normal weight at both time-points. There were no significant differences in measured cytokine levels between normal weight and owt/ob groups at age 8 years.

Similar to adult studies and some studies in children [11, 12, 28], we found raised IL-6 levels in overweight girls at 15 years. Adipose tissue secretes 10–35% of circulating IL-6 plasma levels in healthy humans, and IL-6 plasma concentrations are associated with the development of the metabolic syndrome, type 2 diabetes and cardiovascular disease [29]. We also found raised IL-8 in owt/ob girls at 15 years. This was earlier reported by Herder et al. [12] who found a correlation between IL-8 and waist circumference (adjusted for sex and age) but not BMI in German adolescents aged 15 years. IL-8 levels correlate with an increased risk of future cardiovascular disease in healthy adults, and IL-8 levels are elevated in adults with unstable coronary artery disease [30, 31].

As we have found in overweight girls at 15 years, elevated IL-10 levels have also been reported in other inflammatory conditions [32, 33], and may indicate attempts to inhibit inflammation by downregulating the secretion of proinflammatory cytokines [34–38]. Recent findings suggest that elevated IL-10 levels are beneficial in patients with acute coronary syndromes, particularly in those with elevated CRP levels [39–43].

Despite finding raised IL-6, IL-8 and IL-10 levels in owt/ob girls at 15 years, we found no significant differences in children aged 8 years. Previous studies have shown raised CRP, IL-6 and TNF-α and decreased adiponectin levels in owt/ob children as young as 6 years of age [9, 11, 44, 45]. Yoshida et al. [46] reported a significant

Table 2. BMI groups and cytokine levels at 15 years

	Acceptable BMI (n = 68)	BMI gainers (n = 22)	BMI losers (n = 7)	Overweight/obese (n = 21)	p value
IL-6, pg/ml	8.3 (0–9.2)	9.0 (6.8–12.0)	7.2 (0–9.2)	7.9 (6.6–9.5)	0.091
IL-8, pg/ml	18.9 (11.6–27.3)	25.0 (22.3–30.1)	18.4 (14.5–28.3)	22.8 (14.3–30.3)	0.065
IL-10, pg/ml	7.8 (0.9–7.9)	7.9 (6.0–8.2)	7.5 (0.5–7.7)	7.8 (4.1–8.1)	0.047

Participants were grouped into: (1) acceptable BMI children who were not classified as overweight/obese at 8 or 15 years; (2) BMI gainers – children with acceptable BMI at 8 years, but overweight/obese at 15 years; (3) BMI losers – children who were overweight/obese at 8 years, but had an acceptable BMI at 15 years, and (4) overweight/obese at both time-points – children who were overweight/obese at 8 and 15 years. BMI groups were determined using IOTF cut-points [21]. p values were determined using Kruskal-Wallis test.

association between CRP and BMI z-scores in Japanese children aged 7–10 years. Chu et al. [44] reported raised CRP levels in Taiwanese children aged 12–15 years, but did not adjust for the effects of age and sex on BMI. Interestingly, despite seeing no differences in cytokine levels between normal weight and owt/ob children at 8 years, owt/ob children at 8 years had raised HOMA-IR compared to normal weight children, suggesting that measures of insulin resistance may occur before changes in cytokine levels.

In the current study, we observed that cytokine levels changed with changes in weight status. We found raised levels of IL-8 and IL-10 in girls who changed weight status from normal weight to owt/ob between age 8 and 15 years compared to those who remained normal weight at both time-points. In both boys and girls, IL-10 levels were lower in the group that changed from owt/ob to normal weight over the same time period. Similarly, Reinehr et al. [47] found decreased CRP and TNF-α levels in obese children aged 9–13 years who had a substantial weight loss (decrease in BMI z-score $\geq$0.5) after 1 year. Our findings, albeit with a small sample size, suggest that cytokine regulation during childhood may be influenced by changes in weight status. Future studies with larger sample sizes are needed to confirm this finding.

The differences in cytokine levels between normal weight and owt/ob children at 15 years were only seen in girls. Taken with the lack of significant differences in cytokine levels between normal weight and owt/ob groups at 8 years, we speculate on the potential role of pubertal changes of hormones in influencing changes in cytokine levels with weight status, although our results did not change after adjustment for pubertal status. Self-assessed Tanner pubertal staging may not be a reliable measure for puberty, with evidence that 40% of girls and boys rate their pubertal status incorrectly [48], hence these results need to be interpreted with some caution. Nevertheless, pubertal status has been shown to be associated with cytokines in both animal and human studies [10, 49, 50]. In a mouse study, Lamason et al. [49] found that innate immune response genes were highly upregulated in postpubertal male mice, whereas adaptive immune response genes were highly upregulated in postpubertal female mice that also had significantly higher levels of cytokines and chemokine production than postpubertal male mice. Martos-Moreno et al. [50] measured adiponectin and IL-6 levels in 160 Spanish children and found decreased adiponectin levels in mid-pubertal boys compared to girls, similar to findings from Winer et al. [10]. IL-6 levels decreased in both sexes during puberty and were correlated with testosterone and estradiol levels. In addition, visceral fat secretes greater amounts of inflammatory cytokines (adiponectin, IL-6), compared to subcutaneous fat [51]. Direct measures of visceral fat were not included in this study.

These results should also be interpreted in light of the fact that we used systemic measures in our study. Cytokine release usually occurs locally in the affected tissue or organ, before entering the circulation [52]. Therefore, a relatively small increase in circulating cytokine levels may reflect a significant increase in cytokine concentration at the tissue level. Thus, the systemic measures in our study may underestimate the concentrations at a local level and their potential significance in target tissues.

In conclusion, IL-6, IL-8 and IL-10 levels in overweight children vary with age and sex. We found increased IL-6, IL-8 and IL-10 levels in owt/ob girls, but not in boys, at 15 years. No significant differences in levels were seen at 8 years, despite owt/ob children having higher markers of

insulin resistance. These results suggest that obesity-associated inflammation may be influenced by pubertal changes of hormones interacting with the effects of insulin resistance.

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